

VOLATILE, POTENTIAL ATTRACTANTS FROM RIPE COFFEE FRUIT FOR FEMALE MEDITERRANEAN FRUIT FLY

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Abstract—Twenty-eight volatile compounds from freshly crushed, ripe, dark red coffee fruit, *Coffea arabica*, were identified by dynamic headspace analysis techniques. Identifications were made on the basis of a comparison of Kovats indices and GC-MS spectra for unknowns and authentic samples. Of the compounds identified, 10 were alcohols, nine were aldehydes, five were ketones, and four were monoterpenes. The five most abundant volatiles in decreasing order were hexanal (21%), 2-(*E*)-hexenal (11%), 3-methyl-1-butanol (9.0%), 3-methyl-1-butanal (8.5%), and 1-hexanol (8.4%). The five least abundant volatiles of the 28 identified, in increasing order, were decanal (0.19%), methyl hexanoate (0.33%), pulegone (0.44%), α -isomenthone (0.45%), and 2-nonanone (0.55%). In preliminary tests, many of the identified volatiles attracted more female Mediterranean fruit flies than the control.

Key Words—Coffee fruit, *Coffea arabica*, host, Mediterranean fruit fly, *Ceratitis capitata*, medfly, analysis of volatiles, attractant, lure, Diptera, Tephritidae.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a worldwide agricultural pest of fruits, nuts, and vegetables (Hagen et al., 1981; Jackson

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and Lee, 1985). The fly's presence in the continental United States is prevented through vigorous detection, quarantine, and eradication in tropical and subtropical areas where hosts exist (Anonymous, 1996).

C. capitata (medfly) oviposits in more than 300 species of fruits and vegetables (Liquido et al., 1991), but the presumed ancestral host in Africa is *Coffea arabica* L. (Vargas et al., 1995). Keiser et al. (1975) indicated that the bark of *C. arabica* and an unspecified part of *C. robusta* Lind. attract medflies. Waikwa (1979) showed that the largest number of medfly eggs are laid in the mature yellow-red coffee fruit and the smallest number in the mature green fruit. The issue of color is addressed by Takana (1965), who demonstrated that yellow and green colored artificial egg-laying receptacles are equally attractive in stimulating oviposition by lab-reared medflies. Although this latter study did not confirm whether the flies use visual or olfactory senses to locate fruits for oviposition, Bateman (1972) suggested that visual senses are involved.

Vargas and Chang (1991) found that the oviposition of medfly eggs in a bottle containing a hot water extract of canned coffee grounds is significantly greater than when orange, guava, or papaya juice is used. Our group (D.R.L., E.B.J., and D.O.M., unpublished data, 1996) found that the odor of crushed ripe coffee fruit is more attractive for female medflies than males in flight-tunnel choice tests; the odor of coffee produced a 2.5-fold increase in the number of female landings on fruit models for oviposition. Traps baited with crushed coffee fruit in release-recapture tests captured 15–30 times greater numbers of flies than did unbaited traps. In preliminary release-recapture tests, bucket-type traps baited with fresh versus previously frozen (-80°C) ripe coffee fruit captured similar numbers of *C. capitata*. In the open field, there was a very quick aggregation of flies on leaves that had been rubbed with the inside of the fresh coffee fruit skins.

Prokopy and Vargas (1996) showed that the odor of intact or crushed coffee fruit is more attractive to both sexes of medflies compared to lower-ranking hosts and nonhosts when exposed to laboratory wind-tunnel and field conditions. The odor of recently crushed ripe coffee fruit is more attractive than that of intact ripe fruit and unripe fruit. Mature females carrying a high egg load are more attracted to the odor of ripe coffee fruit than are immature females. Mature laboratory-cultured female medflies and mature wild-origin female medflies give similar response patterns to coffee fruit.

Candidate female medfly attractants isolated from the odor of freshly crushed, ripe coffee fruit, in conjunction with male medfly produced pheromones (Jang et al., 1994), protein baits, and parapheromones (Beroza et al., 1961; Sivinski and Calkins, 1986; McGovern and Cunningham, 1987, 1988) have potential for monitoring and capturing this economic pest. This paper describes the identification of volatile, potential attractants for female medflies from freshly crushed, ripe, coffee fruit.

METHODS AND MATERIALS

Fruit Collection and Storage. Ripe, dark red coffee fruit, *Coffea arabica* L., Blue Mountain variety, was collected March 7, 1995, from a small commercial grove in Haleiwa Oahu, Hawaii. The fruit was held at -80°C for one week to kill any possible larvae that might have been present so that exportation to the continental United States would be permitted. The fruit was then shipped to Beltsville, Maryland, and stored at -10°C until analyses.

Collection of Volatiles. Coffee fruit (50) were removed from the freezer and allowed to defrost for a few minutes to soften. Each fruit was then crushed with tweezers and immediately placed in a three-neck, round-bottom flask. Pre-purified nitrogen (i.e., passed through an activated charcoal bed) was swept at 300 ml/min over the crushed coffee fruit headspace, and volatiles were collected in a glass tube packed with 300 mg of activated charcoal (Darco, 20–40 mesh, Aldrich Chemical Co., Milwaukee, Wisconsin). Activated charcoal was used as the trapping agent because of its high efficiency to adsorb a variety of organic compounds (Heinz et al., 1966). The charcoal used in the collection trap was prepurified by continuous extraction (Soxhlet extractor) with methylene chloride and then benzene (CAUTION: benzene and methylene chloride, suspected cancer-causing agents, should be handled with care and with adequate ventilation). After collecting volatiles for 15 hr, the charcoal trap was removed and eluted with ca. 0.3 ml of methylene chloride. The methylene chloride was analyzed without concentration. The efficiency of the charcoal trap to collect volatiles was determined by inserting a second charcoal trap (equal size and load) in the purge stream after the first trap. Gas chromatographic (GC) analysis of the eluate from the second trap showed complete absence of any volatiles associated with the coffee fruit. The collection of volatiles was replicated three times using freshly crushed coffee fruit each time.

Gas Chromatography (GC). A Shimadzu model GC-9 (Shimadzu, Columbia, Maryland) equipped with a flame ionization detector (FID) and a bonded DB-1 (J&W Scientific, Folsom, California) fused silica capillary column (60 m \times 0.248 mm ID, 0.25- μm film thickness) was used to analyze volatile components from each replicate collection. GC peak areas were quantified using a Shimadzu CR-4A Integrator. GC operating conditions: injector/detector temperature, 280°C ; helium carrier, ca. 1 ml/min (4 kg/cm² head pressure), injector operated in split mode, 175:1; temperature program, 50°C to 250°C at $5^{\circ}\text{C}/\text{min}$.

Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS was performed on a Hewlett-Packard 5890A-GC-MS equipped with a 5971A MSD and an HP5 (Hewlett-Packard, Avondale, Pennsylvania) bonded fused-silica capillary column (25 m \times 0.2 mm ID, 0.11- μm film thickness) for each replicate collection. GC conditions were the same as those described for GC analysis on

the Shimadzu instrument except that the injection port was operated in the splitless mode. MS conditions (EI mode) were: ionization voltage, 70 eV; mass range, m/z 30–550; ion source temperature, 180°C. The mass spectra of the unknown compounds were compared with those in the Wiley/NBS spectral data base. Identifications were also made by comparing Kovats indices (KI) of unknowns with those determined for authentic samples (Kovats, 1966).

Insect Rearing, Bioassay, and Statistics. Laboratory-reared medfly pupae were obtained from the USDA-ARS, Tropical Fruit & Vegetable Research Laboratory rearing facility, Honolulu, Hawai. Flies were allowed to emerge in a screen cage containing sugar, water, and hydrolyzed protein. They were kept at 24–26°C, 60–70% relative humidity, and 12L:12D cycle. At five to eight days after emergence, cages were placed in a cold room where females were put into groups of 50 females in plastic containers (11.5 cm in diameter, 7.5 cm deep) with nylon mesh covers and containing sugar, water, and hydrolyzed protein. Flies used for testing were held for 24 hr at room temperature.

Bioassays were conducted in a flight tunnel (Jang and Light, 1991), 0.9 m \times 0.9 m \times 2.8 m. Fifty previously frozen coffee fruit were crushed and placed in a one-quart mason jar outside of the tunnel. A second one-quart mason jar was left empty. Compressed air was pumped into the jars, and the headspace odor from the jars was pumped via Teflon tubing into the tunnel. The Teflon tubing from each jar was attached to a panel trap. The panel trap was made out of a dark green file folder (14 cm \times 23 cm) with a 6-cm hole cut 1 cm from the bottom. The front and back of the panel were coated with Tanglefoot sticky glue (Tanglefoot Co., Grand Rapids, Michigan). The odor chamber was made using a 250-ml plastic cylindrical bottle (Nalgene), with the bottom third cut off and a hole drilled into the cap to insert the Teflon tubing. The odor chamber was placed through the hole in the panel. The flow rate of the air into the cylindrical bottle was 200 ml/min.

For each assay, 50 mated females (5–8 days old) were released at the back of the tunnel and allowed to fly within the tunnel for 1 hr. The number of females captured on the sticky panel was recorded. Data were analyzed using PROC TTEST (SAS Institute, 1988).

RESULTS AND DISCUSSION

The constituents of the presumed ancestral host, *Coffea arabica* L., (Vargas et al., 1995) are of interest in understanding the complex life cycle of the medfly. In preliminary release–recapture tests, bucket-type traps baited with fresh versus previously frozen (–80°C) ripe coffee fruit captured similar numbers of *C. capitata* (D.R.L., E.B.J., and D.O.M., unpublished data, 1996). The same observations with female medflies were made for fresh versus previously frozen

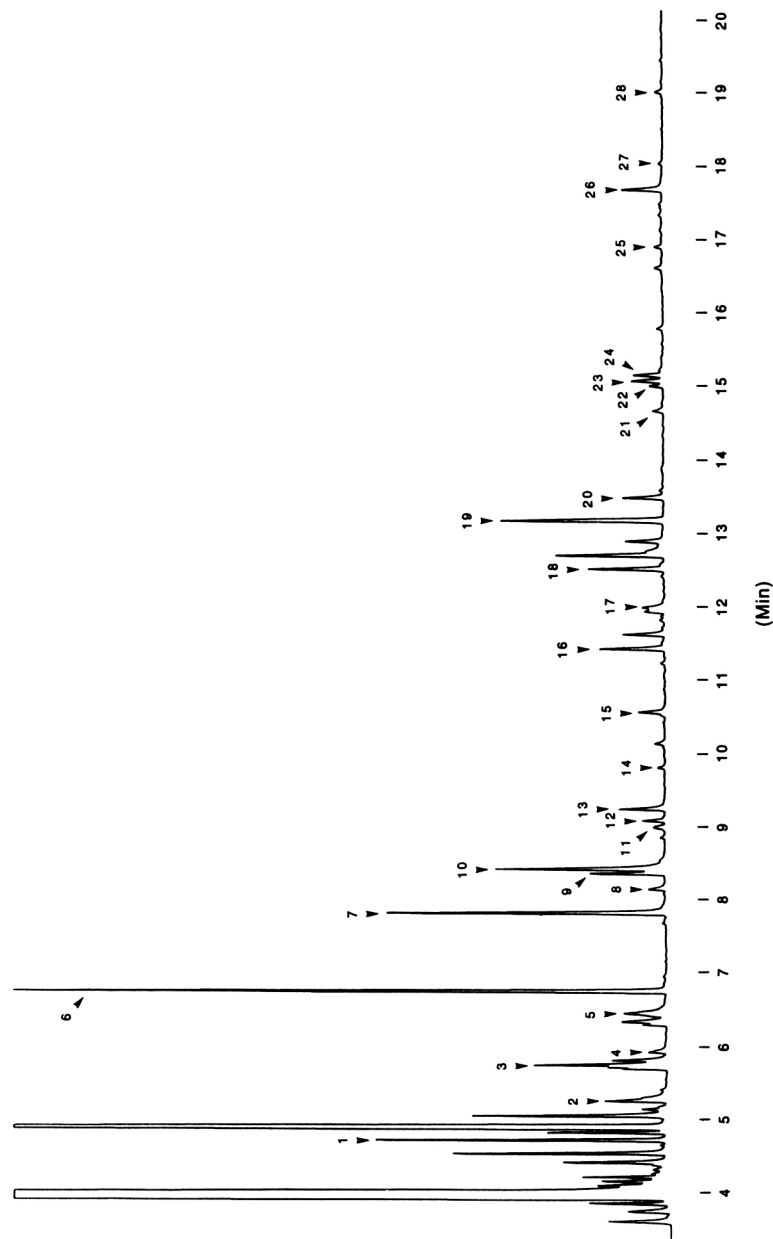


FIG. 1. Gas chromatogram from a temperature programmed analysis of the volatile components from 50 freshly crushed, ripe, dark red coffee fruit, *Coffea arabica*. Peak numbers correspond to peak numbers in Table 1. See Table 1 for MS data, Kovats indices, relative area, and flight tunnel tests.

TABLE 1. VOLATILES IDENTIFIED FROM 50 CRUSHED, RIPE DARK RED COFFEE FRUITS, *Coffea arabica* BLUE MOUNTAIN VAR.

GC peak ^a	Compound	Mass spectral ions, <i>m/z</i>		Ref. KI ^b	Relative area (%)	Female medflies (N) ^d	Control female medflies (N)
		M ⁺ (intensity)	Base peak				
1	3-methyl-1-butanol	86 (8)	44	633	8.5	12	1
2	benzene ^c	86 (1)	44	674	4.0	0	0
3	pentanal	ND ^f	55	724	9.0	9	2
4	3-methyl-1-butanol	84 (56)	55	727	0.88	1	1
5	2-(E)-pentenal	86 (13)	57	760	3.6	10	1
6	2-(Z)-pentenal	100 (1)	44	776	21	3	3
7	hexanal	98 (16)	41	827	11	0	1
8	2-(E)-hexenal	100 (3)	41	839	0.95	9	0
9	3-(Z)-hexenal	100 (3)	57	849	3.2	12	0
10	2-(E)-hexenal	ND	56	852	8.4	1	0
11	1-hexanol	114 (5)	43	874	0.70	5	1
12	2-heptanone	112 (4)	41	879	0.92	0	0
13	4-(Z)-heptenal	ND	45	886	2.1	7	0
14	2-heptanol	130 (0.2)	74	908	0.33	3	0
15	methyl hexanoate	106 (95)	77	933	1.5	1	3
16	benzaldehyde	ND	57	964	3.2	2	0
17	1-octen-3-ol	ND	59	985	1.7	6	1

18	1-methoxy-4-methylbenzene	122 (53)	91	1003	1003	3.3	1	0
19	2-cyclohepten-1-one	110 (21)	81	1020	1024	7.7	1	2
20	2-(<i>E</i>)-octenal	126 (1)	55	1035	1035	1.9	0	4
21	2-nonanone	142 (7)	58	1073	1074	0.55	3	3
22	α -terpinolene	136 (68)	71	1083	1085	0.55	3	1
23	linalool	ND	71	1087	1087	1.3	1	0
24	phenethyl alcohol	122 (33)	91	1089	1089	1.2	3	4
25	α -isomenthone	154 (25)	112	1148	1148	0.45	1	0
26	methyl 2-hydroxybenzoate	152 (48)	120	1175	1175	2.1	3	1
27	decanal	ND	43	1187	1187	0.19	8	3
28	pulegone	152 (56)	81	1221	1221	0.44	4	5

^aPeak numbers correspond to those in Figure 1.

^bRef. Kovats indices calculated from retention time data of authentic sample obtained on a DB-1 capillary column.

^cKovats indices calculated from retention time data on a DB-1 capillary column.

^dFlight-tunnel tests with 5 μ l volatile compound on 4.25 cm filter paper in half-size Jackson traps; 100 female flies (5–8 days old) were released and trap captures were recorded after 2 hr.

^eA contaminant from the prepurification of the charcoal.

^fND = not detected.

guava fruits, *Psidium guajava* L., another host of medfly. Flight-tunnel responses of female medflies to the odor of crushed coffee fruit that had been previously frozen showed a clear preference to the coffee over clean air in dual choice assays. The response of the flies to coffee volatiles [13.7 (\pm 1.8) flies] was significantly greater than to the clean air control [2.8 (\pm 0.8) flies] [$P < 0.05$]. The presence of volatile compounds attractive to gravid female medflies in the flight-tunnel assays suggested that one or more of the compounds identified in this study may be responsible. The response noted in this study was comparable to the results of flight tunnel responses of female flies to the odor of freshly crushed (non-frozen) fruit (D.R.L., E.B.J., and D.O.M., unpublished data, 1996).

In preliminary tests, several identified compounds appear more attractive to female medflies than other compounds when compared to a control (Table 1). Further testing of the individual compounds is currently being carried out to try to determine the most attractive compounds.

A typical GC profile of the volatiles purged from 50 freshly crushed, ripe, dark red coffee fruit, *C. arabica*, is shown in Figure 1. Chemical identities are shown in Figure 1 and listed in Table 1 with the Kovats indices (KI) and relative concentrations. Excluding benzene (a contaminant from the prepurification of the charcoal), a total of 28 peaks appearing in the chromatogram of the volatiles were identified on the basis of a comparison of the KI values and GC-MS spectra with those obtained from authentic samples. Four chemical classes are represented by the 28 identified compounds. The class and number of compounds corresponding to that class were: alcohols (10), aldehydes (9), ketones (5), and monoterpenes (4). The five most abundant volatiles in decreasing order were hexanal (21%), 2-(*E*)-hexenal (11%), 3-methyl-1-butanol (9.0%), 3-methyl-1-butanal (8.5%), and 1-hexanol (8.4%). The five least abundant volatiles of the 28 identified, in increasing order, were decanal (0.19%), methyl hexanoate (0.33%), pulegone (0.44%), α -isomenthone (0.45%), and 2-nonanone (0.55%).

Of the 28 compounds identified in freshly crushed, ripe, dark red coffee fruit, 57% are reported as volatiles in fresh, ripe guava fruits, which also attract medflies. Pentanal, 2-(*E*)-pentenal, 2-(*Z*)-pentenol, 1-octen-3-ol, terpinolene, and decanal (Idstein and Schreier, 1985); hexanal and 3-(*Z*)-hexenol (Chyau et al., 1992; Idstein and Schreier, 1985; Nishimura et al., 1989; MacLeod and Gonzalez De Troconis, 1982); 2-(*E*)-hexenal, 2-(*E*)-hexenol, and 1-hexanol (Chyau et al., 1992; Idstein and Schreier, 1985; Nishimura et al., 1989); methyl hexanoate (Idstein and Schreier, 1985; MacLeod and Gonzalez De Troconis, 1982); benzaldehyde (Idstein and Schreier, 1985; Nishimura et al., 1989; MacLeod and Gonzalez De Troconis, 1982); phenylethyl alcohol (Idstein and Schreier, 1985; Nishimura et al., 1989); 3-methyl-1-butanol (MacLeod and Gonzalez De Troconis, 1982); and linalool (Nishimura et al., 1989) are also observed as constituents of fresh, ripe guava fruit.

The latter compound, linalool, is one of the volatiles produced in the male medfly that elicits responses from female medfly antennae (Cossé et al., 1995). Baker et al. (1990) also tested this compound as a component of the male medfly sex pheromone and found that it caught 23 times more flies than controls.

The presence of the commonly reported members of the green leaf volatile complex [hexanol, 2-(*E*)-hexenol, 3-(*Z*)-hexenol, hexanal, and 2-(*E*)-hexenal], except for 3-(*Z*)-hexenal (Dickens et al., 1990), among the 28 identified volatile components of freshly crushed, red, ripe coffee fruit is of considerable interest because this complex enhances the pheromone response of the medfly. Green leaf volatiles increase the number of landings made by female flies on the odor source relative to male odor alone. The single green leaf volatile alone, 2-(*E*)-hexenal, also enhances response of female medflies to male odor.

The isolation and identification of headspace volatiles of the primary host of medflies has added more data to the complex problem of developing an effective lure for the female Mediterranean fruit fly.

CONCLUSIONS

In summary, we have identified 28 volatile compounds present in freshly crushed, ripe, dark red coffee fruit, *C. arabica*, the primary host of Mediterranean fruit flies. Four classes of compounds were identified with most volatiles being either alcohols or aldehydes. Five of the six components of the green leaf volatile mixture (Dickens et al., 1990) were present in the volatiles, and one of the reported constituents of the male medfly sex pheromone complex (Cossé et al., 1995) was present. In preliminary tests, several identified compounds appear more attractive to female medflies than other compounds when compared to a control. Further testing of the individual compounds is currently being carried out to determine the most attractive compounds. We hope that information from this study and future studies will contribute to the development of effective lures for the female Mediterranean fruit fly.

REFERENCES

- ANONYMOUS. 1996. <http://www.aphis.usda.gov/oa/medfly.html>
- BAKER, P. S., HOWSE, P. E., ONDARZA, R. N., and REYES, J. 1990. Field trials of synthetic sex pheromone components of the male Mediterranean fruit fly (Diptera: Tephritidae) in southern Mexico. *J. Econ. Entomol.* 83:2235-2245.
- BATEMAN, M. A. 1972. Ecology of fruitflies. *Annu. Rev. Entomol.* 17:494-518.
- BEROZA, M. GREEN, N., GERTLER, S. I., STEINER, L. F., and MIYASHITA, D. M. 1961. New attractants for the Mediterranean fruit fly. *J. Agric. Food Chem.* 9:361-365.
- CHYAU, C.-C., CHEN, S.-Y., and WU, C.-M. 1992. Differences of volatile and nonvolatile constituents between mature and ripe guava (*Psidium guajava* Linn) fruits. *J. Agric. Food Chem.* 40:846-849.

- COSSÉ, A. A., TODD, J. L., MILLAR, J. G., MARTÍNEZ, L. A., and BAKER, T. C. 1995. Electroantennographic and coupled gas chromatographic-electroantennographic responses of the Mediterranean fruit fly, *Ceratitis capitata*, to male-produced volatiles and mango odor. *J. Chem. Ecol.* 21:1823-1836.
- DICKENS, J. C., JANG, E. B., LIGHT, D. M., and ALFORD, A. R. 1990. Enhancement of insect pheromone responses by green leaf volatiles. *Naturwissenschaften* 77:29-31.
- HAGEN, K. S., ALLEN, W. W., and TASSAN, R. L. 1981. Mediterranean fruit fly: The worst may be yet to come. *Calif. Agric.* 35:5-7.
- HEINZ, D. E., SEVENANTS, M. R., JENNINGS, W. G. 1966. Preparation of fruit essences for gas chromatography. *J. Food Sci.* 31:63-68.
- IDSTEIN, H., and SCHREIER, P. 1985. Volatile constituents from guava (*Psidium guajava*, L.) fruit. *J. Agric. Food Chem.* 33:138-143.
- JACKSON, D. S., and LEE, B. B. 1985. Medfly in California 1980-1982. *Bull. Entomol. Soc. Am.* 31:29-37.
- JANG, E. B., and LIGHT, D. M. 1991. Behavioral responses of female oriental fruit flies to the odor of papayas at three different ripeness stages in a laboratory flight tunnel (Diptera: Tephritidae). *J. Insect Behav.* 4:751-762.
- JANG, E. B., LIGHT, D. M., BINDER, R. G., FLATH, R. A., and CARVALHO. 1994. Attraction of female Mediterranean fruit flies to the five major components of male-produced pheromone in a laboratory flight tunnel. *J. Chem. Ecol.* 20:9-20.
- KEISER, I., HARRIS, E. J., MIYASHITA, D. H., JACOBSON, M., and PERDUE, R. E., JR. 1975. Attraction of ethyl ether extracts of 232 botanicals to oriental fruit flies, melon flies and Mediterranean fruit flies. *Lloydia* 38:141-152.
- KOVATS, E. 1966. Gas chromatographic characterization of organic substances in the retention index system, in J. C. Giddings and R. A. Keller (eds.). *Advances in Chromatography*, Vol. 1. Marcel Dekker, New York.
- LIQUIDO, N. J., SHINODA, L. A., and CUNNINGHAM, R. T. 1991. Host plants of the Mediterranean fruit Fly: An annotated world review. *Misc. Publ. Entomol. Soc. Am.* 77:1-52.
- MACLEOD, A. J., and GONZALEZ DE TROCONIS, N. 1982. Volatile flavour components of guava. *Phytochemistry* 21:1339-1342.
- MCGOVERN, T. P., and CUNNINGHAM, R. T. 1987. New medfly attractants: Halogen analogs of trimedlure. National Conference of the Entomological Society of America, Boston, Massachusetts, Paper #1283. November 29-December 3.
- MCGOVERN, T. P., and CUNNINGHAM, R. T. 1988. Persistent attractants for the Mediterranean fruit fly, the method of preparation and method of use. US Patent 4,764,366. Issued August 16.
- NISHIMURA, O., YAMAGUCHI, K., MIHARA, S., and SHIBAMOTO, T. 1989. Volatile constituents of guava fruits (*Psidium guajava* L.) and canned puree. *J. Agric. Food Chem.* 37:139-142.
- PROKOPY, R. J., and VARGAS, R. I. 1996. Attraction of *Ceratitis capitata* (Diptera: Tephritidae) flies to odor of coffee fruit. *J. Chem. Ecol.* 22:807-820.
- SAS Institute. 1988. SAS/STAT User's Guide. Release 6.03 edition. SAS Institute, Cary, North Carolina.
- SIVINSKI, J. M., and CALKINS, C. 1986. Pheromones and parapheromones in the control of Tephritids. *Fla. Entomol.* 69:157-168.
- TAKANA, N. 1965. Artificial egg laying receptacles for three species of Tephritid flies. *J. Econ. Entomol.* 58:177-178.
- VARGAS, R. I., and CHANG, H. B. 1991. Evaluation of oviposition stimulants for mass production of melon fly, oriental fruit fly, and Mediterranean fruit fly. *J. Econ. Entomol.* 84:1695-1698.
- VARGAS, R. I., WALSH, W. A., and NISHIDA, T. 1995. Colonization of newly planted coffee fields: Dominance of Mediterranean fruit fly over oriental fruit fly. *J. Econ. Entomol.* 88:620-627.
- WAIKWA, J. W. 1979. Ovipositional behaviour of *C. capitata* with reference to coffee berry age. *Kenya Coffee* 44:23-27.